Open Access
J. Glob. Innov. Agric. Sci., 2026, 14(1): 115-120
ISSN (Online): 2788-4546; ISSN (Print):2788-4538
DOI: https://doi.org/10.22194/JGIAS/26.1717
http://www.jgiass.com

Comparative Study of Different Pretreatments on Seed Germination of *Juniperus communis* L.

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Coniferous plants are generally resilient to external environmental conditions and soil types, and they offer a range of ecological and aesthetic benefits. Among coniferous species, Juniperus communis holds a prominent place due to its popularity and suitability for cultivation in the climatic conditions of Azerbaijan. However, its propagation remains challenging due to low germination rates, largely attributed to physiological dormancy associated with its Mediterranean origin, where seeds have adapted to germinate under specific cool and moist conditions. Physical treatments included not only sandpaper abrasion but also exposure to extreme high (60°C) and low (freezer) temperatures. The effectiveness of these pretreatments was assessed using germination parameters such as germination percentage (GP), mean germination rate (MGR), and mean germination time (MGT). Among the tested methods, warm-cold stratification (four weeks at 15°C followed by four weeks at 3°C) yielded the highest germination percentage of 16.7%, significantly outperforming the control (6.7%). In contrast, pretreatments such as prolonged hot water soaking, freezer storage, and mechanical scarification with sandpaper were ineffective, resulting in 0% germination. In conclusion, a comparison with the germination parameters of the control group demonstrated the potential of seed pretreatment as a promising approach for improving conifer propagation and enhancing reproductive outcomes, with warm-cold stratification (15°C for four weeks followed by 3°C for four weeks) emerging as the most effective method.

Keywords: Juniperus communis, seed germination, pretreatment, stratification, scarification.

INTRODUCTION

Coniferous plants are essential for greening efforts because they can thrive in unfavorable environmental conditions and require minimal care (Ahmad et al., 2024). In addition to their decorative qualities, these plants help purify the air by releasing specific compounds that can kill harmful bacteria and viruses, what is very important for improving urban conditions (Rzayeva et al., 2019; Akhmetshin et al., 2024) and also may be used for other purposes (Lapshin et al., 2023). These ecological and practical benefits highlight the importance of increasing the presence of coniferous species in diverse environments, which in turn depends on efficient propagation techniques (Adilbayeva et al., 2021; Guetarni et al., 2022). Seed propagation plays a vital role in the reproduction of coniferous plants, as seeds are a source of genetic variation (Cunia et al., 2024). This is particularly true for *Juniperus Communis* L., which is valued for its unique berry-like cones and evergreen foliage that demonstrate resilience to environmental challenges. However, a notable drawback of seed propagation is the difficulty in breaking seed dormancy in juniper species (Barton, 1951; Clifton et al., 1997; Broome, 2003).

Juniperus Communis L. is a shrub or small evergreen tree, native to Europe, South Asia, and North America, and belongs to family Cupressaceae (Rzayeva et al., 2019). Juniper (*Juniperus Communis* L.) is the species of juniper with the widest range and listed as LC - Least Concern on the International Union for Conservation of Nature (IUCN). This perennial shrub can grow to a height of one to three meters and has a trunk diameter of up to 0.2m. Its foliage is often dark green or gray and has a slow growth rate, typically grows by 10 to 12cm annually. Further morphological details highlight the uniqueness of the species. The branches of *Juniperus Communis* L. are generally red or reddish-brown. Male plants often have a conical-ovate canopy, while female

Rzayeva, A., Gurbanov, E., Mammadova, Z., Huseynova, H., Bayramova, A., Mammadova, M., & Asadova, K. (2026). Comparative Study of Different Pretreatments on Seed Germination of *Juniperus communis* L. *Journal of Global Innovations in Agricultural Sciences, 14*, 115-120.
[Received 21 Feb 2025; Accepted 16 Jun 2025; Published 3Oct 2025]
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plants' canopy branched at the edges. The leaves are needle-shaped, ranging from 11 to 20mm (Pacini & Piotto, 2004) in length and 0.6 to 7.0mm in width, with a triangular shape. They grow in three clusters along the trunk and can remain attached for up to four years (Radoukova et al., 2024). In Azerbaijan, several varieties of common juniper are used in landscaping, with designations based on the morphological characteristics of their c color and shape (Shlapakova et al., 2022). Understanding the reproductive strategy of the species is also crucial when addressing seed dormancy. *Juniperus Communis* L. is a dioecious plant, meaning that individual plants are either male or female (Ward, 2007; Santos et al., 2023). The number of cones produced usually depends on the age of the plant; cones are typically numerous and measure 5 to 9mm in diameter, boasting either an oblong-ovate or spherical shape. Initially, cones appear light green and mature to a blue or dark bluish-black color with a waxy coating. They ripen in the fall of the second or third year. Each cone usually contains three triangular seeds, though in some cases, they may have one or two. Cone development starts in summer with the fertilization of female cones and ends in the next autumn through embryo maturation. Female cones can be found at different stages of maturity on the same plant simultaneously; fruit ripening and dispersal are distributed from October till January (Osborne, 1981; Souravh et al., 2014). Seed germination of *Juniperus Communis* L. is responsive to many environmental factors, including temperature, light, time after dissemination, and soil moisture content (Hajar, 1991; De Luca & Zackrisson, 2007). Temperature is the critical factor that dictates dormancy, maximizes germination percentage and rate, and ultimately determines the success or failure of plant establishment (Heydecker, 1977; El Qadmi et al., 2023; Faraj et al., 2024). The optimal germination temperatures for coastal Mediterranean species are primarily within the range of 5 to 15°C, and these species are characterized by a low germination rate (Wiese & Binning, 1987). The "delay mechanism" refers to a unique survival strategy in which seeds exhibit a low germination rate and thrive only within a specific range of cool temperatures. This adaptation is particularly advantageous for Mediterranean species, allowing them to cope with the unpredictable patterns of rainfall typical of their environment. By delaying germination until conditions are more favorable, these plants increase their chances of survival in a climate where moisture availability can vary greatly (Fenner & Thomson, 2005). Stratification replicates this natural delay by exposing seeds to controlled periods of cold (and sometimes warm) temperatures, thereby breaking dormancy and synchronizing germination with optimal environmental conditions. According to Mala and Cheong (2020), seed dormancy is a crucial adaptation to survive till the environmental conditions become conductive for active growth. For most coniferous plants the low percentage of germination can be also explained with impermeability of seed coat. Thus, scarification, stratification and acid application may be used to break dormancy in hard coated seeds of hard coated coniferous plants (Jones, 1989; Gurbanov & Rzayeva, 2019; Nawrot-Chorabik et al., 2021). In this context, the present study aims to evaluate the effectiveness of various pretreatment methods for breaking seed dormancy and improving the germination rate of Juniperus communis.

MATERIALS AND METHODS

***Samples*:** *Juniperus Communis* L. ripen cones were collected in March 2024 from Khizi region in Azerbaijan [N 40.8811 and E 48.8581]. The altitude of the samples collection place was 859m from the sea level, annual temperature is 11°C and annual precipitation is 530mm.

Samples preparation: We have carefully extracted seeds from cones right after collection. The seeds were then washed under a stream of water. Then according the methodology suggested by Tadeusz Tylkowski (2009) seeds were soaked into the water to separate filled seeds from empty ones that were floating on the surface. Then chosen filled seeds were dried at room temperature for 10 days. After 10 days, dry seeds were weighted and counted. 30 seeds were chosen for each pretreatment.

***Pretreatment methods*:** Juniperus communis seeds were acted upon to 10 sub-pretreatments (Table 1) of 8 main treatments as

1. High temperature treatment: as seeds were soak in hot water for 5 and 20min
2. Low temperature treatment seeds were soak in cold water for 1 day
3. Application of chemicals - for this pretreatment 2% HCl solution has been used
4. High temperature treatment altered with chemicals application - in this pretreatment hot water 60°C (1 hour) treatment was altered with application of 2% HCl solution
5. Warm-cold stratification - in this pretreatment 15 C warm stratification lasted for 4 months altered with 3 C cold treatment lasting for 4 months
6. Mechanical scarification- in this pretreatment seeds were scratched with sand paper
7. Mechanical scarification altered by low temperature treatment
8. Control - no pretreatments.

***Stages of research*:** After 10 sub-pretreatments for Juniperus communis seeds were employed, seeds were placed to Petri dishes with 1 % agar extract. Each day germinated seeds were counted and extracted from the media and in the last 20 days when no additional germination was detected the test was considered as completed. If the tip of radicle is released from the seed coat a seed was considered as germinated (Tylkowski, 2009). Then for each pretreatment method

special germination parameters were counted: germination percentage (GP), mean germination time (MGT) and mean germination rate (MGR).

Table 1. Summary list of pretreatments.

Pretreatment	Description
Pr 1	Soak in hot water 60C (5 min)
Pr 2	Soak in hot water 60C (20 min)
Pr 3	Soak in cold water (1 day)
Pr 4	Soak in HCl solution (2 %)
Pr 5	Soak in hot water 60C (1 hour) then HCl solution (2%)
Pr 6	Warm 15C /cold 3C stratification
Pr 7	Store in freezer 40 days
Pr 8	Scratch with sand paper
Pr 9	Scratch with sand paper, soak in hot water 60C (20 min) and store in freezer 40 days
Pr 10	Control (no pretreatment)

***Parameters of investigation*:** Germination percentage has been calculated according the equation:

-Germination percentage formula is as follows:

$$\text{Germination \%} = \left(\frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown for germination}}\right) \times 100$$

-Mean germination time and mean germination rate were calculated according the formula described by Ranal et al. (2009):

$$\bar{t} = \frac{\sum_{i=1}^{k} n_i t_i}{\sum_{i=1}^{k} n_i}$$

where t_i- time from the start of the experiment to the i-th observation (day for the example); n_i: number of seeds germinated in the i-th time (not the accumulated number, but the number correspondent to the i-th observation), and k- last time of germination. Mean germination rate (MGR) is calculated as the reciprocal of the mean germination time.

***Data analysis*:** Before performing Duncan's Multiple Range Tests (International union for the protection of new varieties of plants, 2007), we have applied Analysis of Variance (ANOVA) in Microsoft Excel (2016) for determination of significant differences between the treatment means.

RESULTS AND DISCUSSION

The conducted research unequivocally demonstrates several methods for breaking seed dormancy of Juniperus communis (Table 2, Figure 1). The comparison of germination rates after each pretreatment reveals that the most successful method resulted in a remarkable germination percentage of 16.7%. This was achieved through a combination of 4 weeks at 15°C followed by 4 weeks at 3°C for stratification. Additionally, soaking the seeds in cold water for 1 day yielded a noteworthy germination rate of 10%. In stark contrast, several pretreatments proved completely ineffective, with a germination percentage of 0%. Notably, scratching with sandpaper, soaking in hot water at 60 °C for 20 minutes, and storing the seeds in the freezer for 40 days failed to facilitate any germination. The evidence clearly indicates that hot water pretreatment does not effectively break the dormancy of common juniper seeds.

This finding aligns with earlier work by Wu et al. (2024), who demonstrated that cold stratification significantly enhances germination in J. communis, suggesting that breaking physiological dormancy through temperature cycling mimics natural conditions experienced by seeds overwintering in the soil.

Similarly, Harbol et al. (2023) reported that cold treatment was essential for Juniperus virginiana seed germination, and Khalofah (2022) emphasized the need for prolonged chilling to break dormancy in junipers grown in temperate climates. The positive results from cold stratification in our study support these conclusions and highlight the importance of temperature-based dormancy mechanisms, especially in species with Mediterranean or sub-Mediterranean adaptation. In the experiment, control seeds that received no pretreatments displayed a germination rate of just 6.7%. Interestingly, this rate was equivalent to that of seeds that underwent a 40-day pretreatment in the freezer as well as those that were subjected to sandpaper scarification. This suggests that these methods were equally effective, or rather ineffective, in enhancing germination.

Table 2. Summary of various pretreatments effects on GP, MGT and MGR of *Juniperus Communis* L. seeds.

Pretreatment	GP (%)	MGT (day)	MGR
Pr 1-	6.7 ^{ab}	1.4^{a}	0.7 ^{a}
Pr 2	3.3 ^{a}	0.8^{a}	1.3 ^{ab}
Pr 3	10.0 ^{b}	1.9^{b}	0.5 ^{a}
Pr 4	3.3 ^{a}	0.77^{a}	1.3 ^{ab}
Pr 5	3.3 ^{a}	0.9^{a}	1.1 ^{a}
Pr 6	16.7 ^{d}	3.19^{c}	0.3 ^{a}
Pr 7	6.7 ^{ab}	1.2^{b}	0.8 ^{a}
Pr 8	6.7 ^{ab}	1.7^{b}	1.7 ^{c}
Pr 9	0.0 ^{a}	0.0 ^{d}	0.0 ^{b}
Pr 10	6.7 ^{ab}	1.4^{a}	0.7 ^{a}

In each column mean with the same letter are not significantly different at p<0.0001 Duncus Multiple Range Test (DMRT) GP - Germination percentage, MGT- Mean germination time, MGR - Mean germination rate

On the other hand, the manual removal of seed coats did not lead to any significant improvement in the germination rate, indicating that this technique might not be beneficial for enhancing seed viability. Furthermore, when seeds were treated with hot water for 5 minutes, their germination rates remained on par with the control group. However, extending this hot water treatment to 20 minutes had an adverse effect, significantly reducing the germination percentage. This

indicates that while short exposure to hot water may not harm the seeds, prolonged exposure can be detrimental to their ability to germinate.

The process of alternating between cold and warm stratification not only resulted in a remarkably high percentage of seed germination but also caused a significant increase in the average time it took for germination to occur. In contrast, the application of chemical treatments effectively decreased the mean germination time to just 0.8 days, demonstrating a faster onset of sprouting. However, it was noted that soaking seeds in cold water as a pretreatment had an opposite effect, leading to delays in the germination process.

The use of hydrochloric acid (2%) as a chemical scarifier had limited success in our study, achieving only 3.3% germination. Phuyal et al. (2022) observed similarly modest outcomes when applying acid treatment to J. excelsa, suggesting that acid alone is insufficient to overcome dormancy in hard-coated juniper seeds. However, chemical pretreatments did shorten mean germination time (MGT), pointing to partial softening of seed coats that might facilitate radicle emergence under optimal conditions.

Interestingly, soaking in cold water for 24 hours led to a germination rate of 10%, which, although lower than that of stratification, is consistent with findings by Bettoni et al. (2024), who noted improved germination in J. excelsa with hydration pretreatment. This supports the idea that imbibition and rehydration may help activate metabolic processes in dormant seeds.

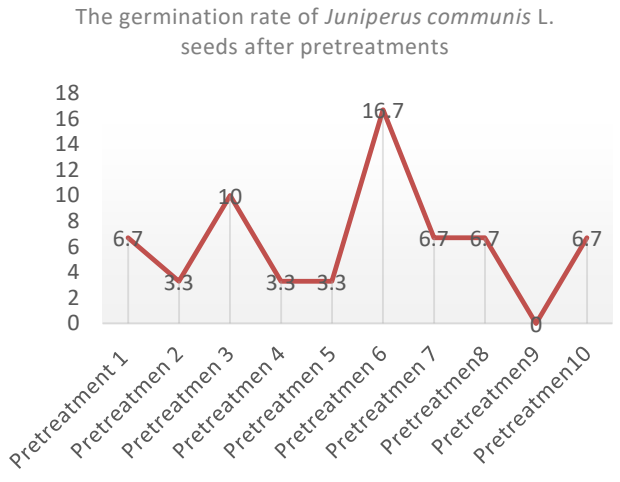

Figure 1. The effectiveness of various pretreatments methods.

The results confirm that J. communis seeds exhibit physiological dormancy typical of Mediterranean and temperate-zone species, requiring a period of cold stratification to trigger germination. While some chemical and hydration-based treatments moderately influenced mean germination time, they did not significantly enhance germination rates, emphasizing the need for environmentally timed cues over solely physical or chemical intervention.

The novelty of this study lies in the systematic comparative evaluation of ten distinct pretreatment methods applied specifically to Juniperus communis seeds collected in the unique climatic and ecological conditions of Azerbaijan. Unlike previous research that focused on isolated methods or different species within the genus, this work offers a comprehensive assessment using standardized metrics (GP, MGT, MGR) and provides a clear recommendation for a regionally adapted, effective stratification protocol.

***Conclusion*:** In this article, different methods to overcome seed dormancy are compared. It was found that alternating between cold and warm stratification results in a high germination percentage, although this method significantly prolongs the average germination time. The study had several limitations, including the application of a limited number of pretreatment methods, as well as the use of a single selected seed preparation technique and one specific set of storage conditions. Future research could focus on investigating combinations of different pretreatment methods, rather than their individual effects, to further enhance germination. Additionally, exploring environmental conditions that may contribute to increased seed germination represents another promising direction.

***CRediT author statement*:** Afaq Rzayeva conceptualized the study and drafted the manuscript. Elshad Gurbanov and Zulfiyya Mammadova conducted the experiments and data analysis. Humira Huseynova and Aynur Bayramova contributed to methodology development and literature review. Mehriban Mammadova participated in seed collection and preparation. Kamala Asadova supervised statistical evaluation and critically revised the manuscript. All authors read and approved the final version of the manuscript.

***Conflict of interest*:** The authors declare that they have no conflict of interest.

***Funding*:** This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

***Ethical statement*:** This study did not involve experiments on animals or humans**.**

***Availability of data and material*:** The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

***Acknowledgment*:** The authors thank editorial of Journal of Global Innovations in Agricultural Sciences for providing review and support within publication process.

***Informed consent*:** N/A

***Consent to participate*:** Not applicable.

***Consent for publication*:** All authors consent to the publication of this manuscript.

***SDGs addressed*:** Climate Action; Life on Land

***Policy referred*:** Forestry and greening policies; national reforestation and afforestation policies; UN Convention on Biological Diversity (CBD) and United Nations Framework Convention on Climate Change (UNFCCC).

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